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Maternal Hormone Levels and Perinatal Characteristics: Implications for Testicular Cancer

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PURPOSE: It was hypothesized that the risk for testicular germ cell tumors (TGCTs) is associated with maternal hormone levels. To examine the hypothesis, some studies used perinatal factors as surrogates for hormone levels. To determine the validity of this assumption, hormone–perinatal factor relationships were examined in the Collaborative Perinatal Project.

METHODS: Maternal estradiol, estriol, and testosterone levels in first- and third-trimester serum samples were correlated with perinatal factors in 300 mothers representative of populations at high (white Americans) or low (black Americans) risk for TGCT.

RESULTS: For white participants, testosterone levels were associated negatively with maternal height ($p < 0.01$) and age ($p = 0.02$) and positively with maternal weight ($p = 0.02$) and body mass index (BMI; $p < 0.01$), whereas estradiol levels were associated negatively with height ($p = 0.03$) and positively with son's birth weight ($p = 0.04$). For black participants, estriol levels were associated negatively with maternal weight ($p = 0.01$), BMI ($p = 0.02$), and gestational age $p < 0.01$) and positively with son's birth weight ($p < 0.01$), length ($p = 0.04$), and head circumference ($p = 0.03$).

CONCLUSIONS: These findings indicate that use of perinatal characteristics as surrogates for hormone levels should be limited to a specific ethnic group. For white men, previously reported associations of TGCT with maternal weight and age may be caused by lower maternal testosterone levels.

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INTRODUCTION

There is increasing evidence that testicular germ cell tumors (TGCTs) originate in utero (1). Although the mechanism that increases risk is unknown, it was suggested that an imbalanced intrauterine hormonal milieu may be important (2–4). However, it is very difficult to study the association between a fetal exposure that is not routinely measured, such as hormone levels, and a disease that occurs decades later. To overcome this problem, some studies examined the relationship between perinatal variables and TGCT under the assumption that perinatal variables are good surrogate measures of in utero hormonal conditions.

To test the validity of this assumption, several studies examined relationships between perinatal factors and maternal hormone levels (5–11). However, some of these studies included hormone samples from only one time in pregnancy or studied members of only one ethnic group. In addition, almost all studies to date included mothers pregnant with both male and female fetuses. Because relationships between perinatal factors and maternal hormones may vary by sex of the fetus or by ethnic group, further scrutiny of these relationships was indicated.

With the goal of informing future studies of TGCTs, relationships between perinatal factors and maternal hormone levels were examined in mothers pregnant with male fetuses. The mothers were selected to represent populations at differing risks for TGCT, white Americans (high risk) and black Americans (low risk), to determine whether perinatal factor–hormone relationships vary by ethnicity. In addition, relationships were examined in samples drawn in the first and third trimesters because these are critical periods for testicular descent, the failure of which is associated strongly with risk for TGCTs (12).

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Selected Abbreviations and Acronyms

TGCT = testicular germ cell tumor
BMI = body mass index
CPP = Collaborative Perinatal Project
DHEAS = dehydroepiandrosterone sulfate

METHODS

Study Population

A detailed description of the study population was reported previously (13). Briefly, 150 pairs of black and white mothers were selected from participants of the Collaborative Perinatal Project (CPP). The CPP cohort study was designed to examine perinatal risk factors for neurologic disorders in offspring (14). Pregnant women were enrolled at 12 medical centers in 11 US cities (Baltimore, Boston, Buffalo, Memphis, Minneapolis, New Orleans, New York (two centers), Philadelphia, Portland, Providence, and Richmond) between 1959 and 1965. At 11 study centers, patients were recruited from the participating university hospital's prenatal care clinic, and in one study center (Buffalo), patients were recruited from 13 participating private medical practices. Methods of participant selection varied across study centers. For example, at Columbia-Presbyterian Medical Center, every sixth woman who potentially was eligible was invited to participate; at Charity Hospital in New Orleans, potentially eligible women were selected if their patient identification number ended in zero; and in Boston, all potentially eligible women were invited to participate. Women were ineligible if they were incarcerated, planning to leave the area or give up the child for adoption, or gave birth on the day they were recruited into the study. Records of the number of women who refused to participate at baseline were not kept, but participation rates were assumed to be high (e.g., the rate was >99% at the Johns Hopkins Center in Baltimore; Janet Hardy, Johns Hopkins University, personal communication, November 2001). Characteristics of women in the sample at registration were essentially the same as those in the sampling frame (14). Four percent of subjects who enrolled were lost to follow-up before delivery.

Mothers donated nonfasting blood samples at approximately 8-week intervals throughout their pregnancies, as well as at delivery and 6 weeks postpartum. All serum samples subsequently have been stored in glass vials at −20°C, with no recorded thaws. Details of all clinic visits were recorded in the study records. Maternal characteristics of interest to the current analysis (age, height, prepregnancy weight, smoking, and socioeconomic index) were obtained from the women at the time of enrollment in the study. Neonatal characteristics (length of gestation, birth weight, birth length, and head circumference) were obtained in the delivery room. Approximately 42,000 women were enrolled in the study, and 55,000 children were born into the study. The children were assessed systematically at regular intervals for the presence of birth defects and other outcomes through the age of 7 years. Follow-up to age 7 years was completed for approximately 75% of subjects born into the study.

Mothers were selected for the current study based on the following criteria: pregnant for the first time, gave birth to a singleton male infant who lived at least 1 year, length of gestation between 26 and 48 weeks, blood samples available from both first and third trimesters, baby's birth weight at least 500 g, and baby had no diagnosis of undescended testes, late descending testes, retractile testes, or other malformations possibly related to maternal hormone levels (i.e., central nervous system and related musculoskeletal, genitourinary, inguinal hernia, hydrocele, and supernumerous nipples). The study was limited to mothers pregnant for the first time because estrogen levels were reported to be greater in first pregnancies (10, 15) and some studies linked TGCT risk to birth order (16, 17).

A total of 162 black and 652 white mothers satisfied the study inclusion criteria. The principal limiting criterion was the availability of first-trimester samples because the median entry time into the study for the entire CPP population was 20 weeks' gestation. In addition, the nulliparity criterion restricted the study group to approximately one third of the entire population. Each of the 162 black mothers was matched to a white mother on the closest blood draw gestational age dates. Matches then were reordered to select the 150 pairs best matched on draw dates. For the 300 mothers selected, gestational ages ranged between 30 and 43 weeks.

Laboratory Assays

Serum hormone levels were assessed at the Reproductive Endocrine Research Laboratory of the University of Southern California Keck School of Medicine (13). Unconjugated estriol, unconjugated estradiol, and testosterone were determined by using well-established validated radioimmunoassay methods that are carried out routinely in the laboratory (18, 19).

Study samples, labeled with unique random identification numbers, were analyzed in 17 batches. Each batch contained four quality-control samples aliquotted from a single blood pool. Intraassay coefficients of variation ranged between 3.7% and 12.6%, whereas interassay coefficients of variation ranged between 4.8% and 13.3%.

Statistical Analysis

Distributions of perinatal characteristics were compared by using chi-square tests. Small-for-gestational age was determined by comparing birth weights for each gestational age in weeks in the CPP with the 10th percentile of birth weight

for gestational age developed by Williams et al. (20) for California white non-Hispanic and black births (R.L. Williams, personal communication, July 1983) that occurred between 1970 and 1976. Tertile categories of birth length, head circumference, and maternal prepregnancy weight were based on the distribution in the entire study population. Hormone ratios were calculated by dividing total estradiol and estriol by total testosterone. Multiple linear regression modeling was used to estimate mean change in hormone levels or hormone ratios for an increase of 1 SD in maternal characteristics, including age, height, prepregnancy weight, body mass index (BMI), and number of cigarettes smoked during pregnancy, adjusting for gestational age at blood draw and socioeconomic index. The adjustment for gestational age at blood draw was used because the variable was used as a matching factor in the study design. Results did not differ between models using original hormone levels and models using log-transformed levels. Results of models using original levels are presented next. The SD for each maternal characteristic was calculated from the entire population. Multiple linear regression modeling also was used to estimate mean change in birth weight, birth length, head circumference, and gestational age for an increase of 1 SD in maternal hormone levels or one-unit change in hormone ratios, adjusting for gestational age at blood draw, age, prepregnancy weight, height, cigarette smoking during pregnancy, and socioeconomic index. Because of pronounced correlations between birth weight and birth length ($r = 0.71$), birth weight and head circumference ($r = 0.64$), and birth length and head circumference ($r = 0.51$), each variable was not adjusted for the other when linear regression models of the relationship between birth size and hormone levels were fit. Interactions were assessed by including product terms between pairs of variables in regression models and then were examined by using *t*-tests. All tests of significance were two sided. Analyses were performed using SAS software, version 8.02 (SAS Institute, Inc., Cary, NC).

TABLE 1. Distributions of maternal and neonatal characteristics in white and black participants

	White (n = 150)		Black (n = 150)		
Characteristics	N	%	N	%	*p*
Birth weight (kg)					
<2.5	1	0.7	9	6.0	**0.02**
2.5–4.0	141	94.0	137	91.3	
>4.0	8	5.3	4	2.7	
Birth weight for gestational age					
Small for gestational age	15	10.0	17	11.3	0.71
Not small for gestational age	135	90.0	133	88.7	
Birth length (cm)					
<50	54	36.2	49	32.9	0.28
50–51	45	30.2	58	38.9	
>51	50	33.6	42	28.2	
Head circumference (cm)					
<34	45	30.2	59	39.6	0.07
34	36	24.2	41	27.5	
>34	68	45.6	49	32.9	
Gestational age (weeks)					
<37	14	9.3	20	13.3	0.27
⩾37	136	90.7	130	86.7	
Maternal age (years)					
<20	32	21.3	77	51.3	**<0.0001**
20–24	88	58.7	59	39.3	
>24	30	20.0	14	9.3	
Maternal height (cm)					
<160	37	24.7	48	32.0	0.09
160–165	65	43.3	47	31.3	
>165	48	32.0	55	36.7	
Prepregnancy weight (kg)					
<53.1	52	34.7	49	32.7	0.91
53.1–58.9	46	30.7	49	32.7	
>58.9	52	34.7	52	34.7	
Maternal smoking during pregnancy					
No	96	64.0	107	71.3	0.17
Yes	54	36.0	43	28.7	
Social economic index (tertile)					
I	10	6.7	85	58.2	**<0.0001**
II	57	38.3	43	29.5	
III	82	55.0	18	12.3	

RESULTS

Black mothers were significantly more likely than white mothers to be younger ($p < 0.0001$), be of lower social economic status ($p < 0.0001$), and have low-birth-weight sons ($p = 0.02$; Table 1). No other significant differences were observed.

As previously reported (13), black mothers had significantly higher levels of estradiol ($p = 0.05$) and testosterone ($p \leqslant 0.01$) in first-trimester samples, but had significantly lower estradiol-testosterone and estriol-testosterone ratios ($p = 0.01$). Conversely, there was no difference in first-trimester estriol levels between groups. In third-trimester samples, testosterone levels were significantly higher ($p < 0.01$) and estradiol-testosterone ($p < 0.01$) and estriol-testosterone ($p < 0.001$) ratios were significantly lower among black mothers.

Examination of associations between maternal characteristics and testosterone levels found that testosterone levels were significantly associated with weight, height, BMI, and age of white mothers, but not black mothers (Table 2). For white mothers, both first- and third-trimester testosterone levels significantly increased with increasing weight and BMI and significantly decreased with increasing height. In addition, there was an inverse association between age and testosterone level that was stronger in third- ($p < 0.02$) than first-trimester samples ($p = 0.13$). Conversely, no maternal characteristic was associated with testosterone levels in black mothers.

TABLE 2. Associations between maternal characteristics and serum hormone concentrations among white and black mothers in first and third trimesters

	White Mothers				Black Mothers				Whites vs. Blacks	
	1st Trimester		3rd Trimester		1st Trimester		3rd Trimester		1st Trim	3rd Trim
Maternal characteristics[§] (per SD)	MC*	p-value[¶]	MC*	p-value[¶]	MC*	p-value[¶]	MC*	p-value[¶]	p-value[¶]	p-value[¶]
Testosterone (ng/ml)										
Pre-pregnancy weight	0.13	**<0.01**	0.12	**0.02**	0.12	0.30	0.04	0.82	0.91	0.93
Height	−0.12	**<0.01**	−0.17	**<0.01**	−0.11	0.33	0.14	0.46	0.91	0.19
BMI	0.13	**<0.01**	0.13	**<0.01**	0.12	0.25	0.03	0.88	0.99	0.45
Age	−0.06	0.13	−0.13	**<0.02**	−0.04	0.72	−0.11	0.58	0.73	0.89
Smoking	−0.004	−0.89	0.02	0.61	−0.07	0.53	0.004	0.98	0.56	0.87
Estradiol (ng/ml)										
Pre-pregnancy weight	0.36	0.12	0.87	0.24	0.01	0.97	−1.04	0.22	0.21	0.23
Height	−0.27	0.27	−1.66	**0.03**	−0.26	0.19	−0.02	0.98	0.69	0.33
BMI	0.34	0.11	1.00	0.14	0.04	0.82	−0.08	0.32	0.29	0.09
Age	−0.16	0.51	−0.38	0.63	0.08	0.70	0.18	0.84	0.54	0.62
Smoking	−0.06	0.75	−0.19	0.77	−0.22	0.29	0.10	0.91	0.59	0.84
Estraiol (ng/ml)										
Pre-pregnancy weight	0.12	0.51	0.42	0.32	−0.04	0.69	−1.43	**0.01**	0.05	**<0.01**
Height	−0.15	0.43	−0.29	0.50	−0.06	0.56	0.27	0.63	0.87	0.87
BMI	0.12	0.46	0.42	0.28	−0.03	0.71	−1.26	**0.02**	0.41	**<0.01**
Age	−0.11	0.55	0.61	0.17	0.02	0.86	−0.17	0.78	0.61	0.17
Smoking	−0.04	0.78	−0.26	0.48	−0.11	0.29	−0.13	0.83	0.71	0.88
Estrdiol/testosterone										
Pre-pregnancy weight	0.15	0.46	−0.53	0.41	−0.07	0.63	−0.04	0.94	0.27	0.89
Height	0.01	0.97	0.94	0.16	−0.04	0.78	−0.57	0.29	0.62	0.11
BMI	0.12	0.51	−0.60	0.32	−0.06	0.69	−0.10	0.85	0.38	0.46
Age	0.07	0.74	1.79	**0.01**	0.15	0.34	0.44	0.43	0.59	0.16
Smoking	−0.06	0.74	−0.13	0.82	−0.07	0.66	−0.14	0.80	0.91	0.94
Estriol/testosterone										
Pre-pregnancy weight	0.06	0.71	−0.41	0.37	−0.05	0.59	−0.31	0.49	0.57	0.33
Height	−0.06	0.73	1.28	**<0.01**	0.001	0.99	−0.35	0.45	0.87	**0.02**
BMI	0.06	0.68	−0.56	0.19	−0.04	0.59	−0.22	0.61	0.49	0.80
Age	−0.01	0.96	2.45	**<0.01**	0.05	0.55	0.22	0.64	0.59	**<0.01**
Smoking	−0.02	0.91	−0.27	0.50	−0.05	0.55	−0.22	0.65	0.76	0.85

[§]Each maternal characteristic was rescaled by dividing its standard deviation which was calculated from the entire population.
*Mean change per standard deviation of each maternal characteristics.
[¶]Adjusted for gestational age of blood draw, maternal social economic index, and other maternal characteristics listed in this table.

No significant relationships were observed in either white or black mothers between maternal characteristics and first-trimester levels of estradiol or estriol or ratios of estradiol to testosterone and estriol to testosterone (Table 2). However, several significant differences were noted in third-trimester samples. For white mothers, estradiol levels significantly decreased with increasing height ($p = 0.03$), whereas for black mothers, estriol levels significantly decreased with increasing weight ($p = 0.01$) and BMI ($p = 0.02$). Associations between estriol level and weight and estriol level and BMI in black mothers significantly differed from those in white mothers ($p = 0.01$). For white mothers, higher estradiol-testosterone ratios were associated significantly with increasing age ($p = 0.01$), whereas higher estriol-testosterone ratios were associated significantly with both increasing age ($p < 0.01$) and height ($p < 0.01$). Both associations with estriol-testosterone ratios were significantly different between white and black mothers. Cigarette smoking was not associated with any hormone at any time in black or white mothers (Table 2).

Examination of neonatal characteristics and testosterone levels uncovered no associations (Table 3). However, estradiol levels were associated positively with birth weight and length and negatively with gestational age in white sons. Associations with length and gestational age were much stronger in first- (length, $p = 0.01$; gestational age, $p < 0.01$) than third-trimester samples (length, $p = 0.24$; gestational age, $p = 0.45$). For black sons, estradiol levels were associated negatively with gestational age in both first- ($p < 0.01$) and third-trimester ($p < 0.01$) samples.

For black sons, estriol level was associated positively with birth weight and length and negatively with head circumference and gestational age (Table 3). All associations except that with gestational age were stronger in third-trimester samples. Conversely, for white sons, estriol level was not related to birth weight, length, or head circumference and was

TABLE 3. Associations between neonatal characteristics and maternal serum hormone concentrations among white and black sons in first and third trimesters

	White Mothers				Black Mothers				Whites vs. Blacks	
	1st Trimester		3rd Trimester		1st Trimester		3rd Trimester		1st Trim	3rd Trim
Hormone[§] (per SD)	MC*	p-value[¶]	MC*	p-value[¶]	MC*	p-value[¶]	MC*	p-value[¶]	p-value[¶]	p-value[¶]
Testosterone										
Birth weight (grams)	54.02	0.48	75.44	0.37	−10.69	0.72	−7.36	0.80	0.29	0.38
Birth length (cms)	0.11	0.82	−0.15	0.78	−0.09	0.55	−0.24	0.13	0.54	0.89
Head circumference (cms)	−0.26	0.36	−0.02	0.95	−0.05	0.63	−0.11	0.23	0.63	0.67
Gestational age (weeks)	−0.39	0.36	0.56	0.20	0.001	0.99	−0.04	0.82	0.62	0.14
Estradiol										
Birth weight (grams)	83.60	**<0.01**	73.62	**0.04**	43.91	0.35	52.73	0.16	0.90	0.68
Birth length (cms)	0.46	**0.01**	0.26	0.24	−0.09	0.72	0.16	0.43	0.40	0.64
Head circumference (cms)	0.10	0.37	0.15	0.25	0.05	0.75	0.03	0.82	0.98	0.89
Gestational age (weeks)	−0.72	**<0.01**	−0.14	0.45	−1.09	**<0.01**	−0.42	**0.03**	0.10	0.29
Estriol										
Birth weight (grams)	41.45	0.09	73.63	0.07	19.90	0.75	117.72	**<0.01**	0.73	0.18
Birth length (cms)	0.18	0.23	0.21	0.42	0.05	0.87	0.43	**0.04**	0.70	0.14
Head circumference (cms)	0.05	0.57	0.16	0.27	−0.002	0.99	0.28	**0.03**	0.98	0.44
Gestational age (weeks)	−0.40	**<0.01**	−0.32	0.13	−1.54	**<0.01**	−1.00	**<0.01**	**<0.01**	**0.02**
Total estradiol/total testosterone										
Birth weight (grams)	61.76	0.06	21.10	0.41	−15.47	0.78	19.25	0.60	0.72	0.51
Birth length (cms)	0.44	**0.03**	0.20	0.20	−0.14	0.64	0.43	**0.02**	0.40	0.14
Head circumference (cms)	0.22	0.08	0.13	0.17	−0.05	0.79	0.14	0.23	0.42	0.53
Gestational age (weeks)	−0.61	**<0.01**	−0.32	**0.02**	−0.79	**<0.01**	−0.26	0.18	0.60	0.47
Total estriol/total testosterone										
Birth weight (grams)	24.18	0.36	19.18	0.43	−47.65	0.47	35.78	0.22	0.56	0.22
Birth length (cms)	0.17	0.31	0.09	0.55	−0.07	0.84	0.39	**0.01**	0.89	**0.03**
Head circumference (cms)	0.08	0.44	0.12	0.17	−0.11	0.59	0.19	**0.04**	0.54	0.30
Gestational age (weeks)	−0.28	0.05	−0.37	**<0.01**	−0.93	**<0.01**	−0.39	**<0.01**	0.06	0.84

[§]Each hormone level was rescaled by dividing its standard deviation which was calculated separately for the first and third trimesters from the entire population.
*Mean change per standard deviation of each hormone.
[¶]Adjusted for gestational age of blood draw, maternal age at pregnancy, smoking during pregnancy, social economic index, maternal pre-pregnancy weight, maternal height, and gestational age (for birth weight, birth length and head circumference) and birth weight, birth length, head circumference (for gestational age).

related to only gestational age ($p < 0.01$) in first-trimester samples.

For white sons, estradiol-testosterone ratio was associated negatively with gestational age in both first- and third-trimester samples, whereas it was associated positively with birth length in only first-trimester samples (Table 3). For black sons, estradiol-testosterone ratio was associated significantly with birth length in only third-trimester samples and with gestational age in only first-trimester samples.

The ratio of estriol to testosterone was associated negatively with birth length, head circumference, and gestational age for black sons (Table 3). Associations with head circumference ($p = 0.04$) and birth length ($p = 0.01$) were significant in only third-trimester samples. For white sons, estriol-testosterone ratio was related negatively to gestational age in both first- ($p = 0.05$) and third-trimester ($p < 0.01$) samples.

Comparing neonatal associations between white and black sons, only the association between gestational age and estriol level differed significantly in both first- and third-trimester samples (Table 3). The association between length and estriol-testosterone ratio differed significantly between the two groups, but only in third-trimester samples.

DISCUSSION

Stimulated by racial differences in cancer risk, several studies compared hormone levels in black and white mothers (9, 13, 21). Findings of greater testosterone levels in black mothers led to the hypothesis that lower risk for TGCTs in black men may be caused by greater maternal testosterone levels (21). Alternatively, several TGCT studies hypothesized that increased risks associated with such factors as hyperemesis gravidarum (22) and low birth order (15) may link greater maternal estrogen exposure to TGCTs (17, 23). However, one shortcoming of the TGCT studies is that they almost exclusively enrolled white men. Thus, it has not been clear that results can be extrapolated to nonwhite populations. Findings of the current study support this concern in that some of the examined associations between perinatal factors and hormone levels varied between black and white participants.

Why hormone associations would differ by ethnicity is not clear. Because black mothers had significantly greater testosterone levels overall (13), it is conceivable that testosterone associations became apparent only in the lower range of white mothers. The stronger estriol association with perinatal characteristics of black participants is more puzzling because black and white mothers had similar estriol levels. Estriol is produced predominantly by the placenta from dehydroepiandrosterone sulfate (DHEAS) elaborated by the fetal adrenal glands (24). In black sons, the positive association between estriol level and neonatal size (birth weight, length, and head size) may suggest that larger fetuses produce more DHEAS for conversion to estriol. However, maternal estriol levels of black and white participants did not differ, although birth weights of black sons were significantly less than those of white sons. This result may indicate that black fetuses produce more estriol than white fetuses of the same size. Alternatively, it is possible that placentae of black fetuses are more efficient at converting DHEAS to estriol. Thus, the lower risk for TGCTs in black men might be related to one or more of several features; (i) greater maternal testosterone levels, (ii) lower estriol-testosterone ratios, (iii) greater fetal capacity to produce DHEAS, and (iv) greater placental capacity to synthesize estriol. However, the paucity of data for black men with TGCTs makes it difficult to examine these hypotheses.

In white populations, a number of prior studies of hormone levels and perinatal characteristics were conducted. The relationship between maternal body size and estrogen levels in particular was the focus of a number of investigations. Although one study (10) reported a positive association between weight and free estradiol levels, two studies reported negative associations between height and estriol levels (11, 15) and another study found no association between body size and estrogen levels (9). In one study (15), a negative association between body size and estradiol level was found in white, but not Chinese, mothers. Similarly, the current study found negative associations between height and estradiol levels in white mothers and between weight and BMI and estriol levels in black mothers. Examinations of body size and testosterone levels have been fewer. However, the current study's result linking weight in white mothers to testosterone levels is similar to at least one prior investigation (9). Because several recent studies suggested decreased risk for TGCTs associated with increased prepregnancy weight or BMI (23, 25, 26), the current findings are consistent with a protective effect of greater maternal testosterone levels on white sons.

Studies of maternal age and hormone levels reported that mothers younger than 20 years had lower estrogen levels than other mothers (9, 27), whereas the highest estrogen levels are found in mothers between either 20 and 24 years (28) or 20 and 34 years (9). However, the current study's findings of no association between age and estrogen levels are consistent with results of Kaijser et al. (7, 11). The lower testosterone levels in older white mothers in the present study also are consistent with the report of Troisi et al. (9). Because risk for TGCT was reported to increase with increasing maternal age by some (25, 28, 29), the current data suggest that lower maternal testosterone levels may increase risk for TGCTs in white sons.

Positive associations between maternal estriol, estradiol, and estrone levels and birth weight have all been observed previously (7, 8). The findings of the current study, with associations between estradiol level and birth weight in white sons and estriol level and birth weight in black sons, are consistent with these results. The relationship between maternal estrogen levels and birth weight is of particular interest because birth weight was associated with TGCT risk in a number of studies. The majority of studies found that low birth weight is associated with increased risk for TGCTs (17, 23, 25, 28, 30–32). However, several studies (16, 25, 32) reported a U-shaped relationship with risk, whereas several others (26, 33, 34) reported no association. The combined results may suggest there is more than one pathway to develop TGCTs: one associated with high birth weight and a second associated with low birth weight. The current results suggest that the low-birth-weight pathway would be characterized by lower maternal estradiol levels in white populations. Conversely, the high-birth-weight pathway would be characterized by higher maternal estradiol levels. Because the great majority of cryptorchidism studies reported an association between cryptorchidism and low birth weight (35–38), another feature of the low-birth-weight/low-estradiol pathway may be cryptorchidism.

Inverse associations between gestational age and maternal estriol levels were found in both the current study and a prior multiethnic study (15), but not in a study of estriol levels at term (9). In studies that examined the relationship between gestational age and TGCT risk, most found an inverse association (16, 26, 28, 31, 32, 39). Because higher estrogen levels in the first trimester were associated with shorter gestational age in the current study, higher first-trimester estrogen levels may by extension be associated with increased risk for TGCTs in white men. However, given the positive association between first-trimester estradiol level and birth weight, the estradiol–gestational age association may occur simply because larger babies tend to have shorter gestational periods. It also is possible that the apparent association is caused by errors in determining gestation age.

It previously was speculated that cigarette smoking decreases the production rate or levels of total pregnancy estrogens (40). However, studies that examined the association between estriol excretion and maternal tobacco smoking reached inconsistent results, with some suggesting a negative association (41, 42) and others suggesting no association

(43, 44). Because recent studies and the current study found no association between smoking and maternal serum estrogen levels (7, 9), the smoking—estrogen relationship remains unclear. It should be noted that similar to previous studies (44—48), the current study observed a negative association between birth weight and smoking ($\beta = -3.87$; $p = 0.05$) that suggests that the relationship is not mediated by estrogens.

The current study had several advantages, as well as limitations. Major advantages include that the study was conducted prospectively, included blood samples from only mothers pregnant with male fetuses, and included mothers of more than one ethnic group. The evidence suggesting that maternal hormone levels may be affected by fetal sex (9, 45, 46) makes the second advantage critical to the ability to extrapolate results to TGCT studies. Another advantage, the relatively large sample size, permitted sufficient statistical power to study the associations separately in populations at low (black) and high risk (white) for TGCTs (47). In the current study, any possible confounding effect of parity or multiple births on hormone levels also was eliminated. One theoretical limitation of the study is that hormone levels were measured on samples stored for approximately 40 years. However, steroid hormones are fairly robust, as shown by previous studies of freeze-thaw cycles on serum levels in stored samples (49). In addition, the current study found levels equivalent to those reported in other studies examining newly drawn samples (48). Finally, caution should be exercised in interpreting the results because a sizeable number of comparisons were analyzed.

In conclusion, the current study found that maternal hormone associations with perinatal characteristics varied, sometimes significantly, by ethnic group. This finding suggests that extrapolation of perinatal associations with TGCT risk should not be done across ethnic groups. The current study also suggests that based on relationships between maternal characteristics and hormone levels, lower maternal testosterone levels may increase the risk for TGCTs in white men. The implications for black men are not as clear because of lack of data about the relationship between perinatal characteristics and risk for TGCTs in that group. Inclusion of black men in future studies would be of great benefit in understanding the complex relationships between in utero hormone exposures and TGCT risk.

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